PB80-223597

Response of Bush Bean Exposed to Acid Mist

(U.S.) Corvallis Environmental Research Lab., OR

1980

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1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/J-80-034	JOURNAL ARTICLE	PB 80 223597

4. TITLE AND SUBTITLE
Response of Bush Bean Exposed to Acid Mist

5. REPORT DATE
1980

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Ibrahim J. Hindawi, James A. Rea, and William L. Griffis

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Research Laboratory-Corvallis
Office of Research and Development
U.S. Environmental Protection Agency
Corvallis, Oregon 97330

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
SAME

13. TYPE OF REPORT AND PERIOD COVERED
Journal article

14. SPONSORING AGENCY CODE
600/02

15. SUPPLEMENTARY NOTES
Published in American Journal of Botany, Vol. 67, No. 21, pp. 168-171, Feb. 1980.

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-80-034
JOURNAL ARTICLE

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Reprinted from AMERICAN JOURNAL OF BOTANY
Vol. 67, No. 2, February 1980
Made in United States of America

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

Amer. J. Bot. 67(2): 168–172. 1980.

RESPONSE OF BUSH BEAN EXPOSED TO ACID MIST[1]

IBRAHIM J. HINDAWI[2], JAMES A. REA, AND WILLIAM L. GRIFFIS
U.S. Environmental Protection Agency, Corvallis, Oregon 97330

ABSTRACT

Bush bean plants (*Phaseolus vulgaris* L. cv. Contender) were treated once a week for six weeks with simulated acid mist at five pH levels ranging from 5.5 to 2.0. Leaf injury developed on plants exposed to acid concentrations below pH 3 and many leaves developed a flecking symptom similar to that caused by ozone. An adaxial, interveinal bleached area resembling SO_2 injury also developed on some trifoliate leaves at the low pH treatments. Microscopic observation of injured trifoliates indicated that the palisade cells were plasmolyzed and that the chloroplasts lost structural integrity. Reductions in plant weight and chlorophyll content were detected across the pH gradient. Seed and pod growth were reduced at some intermediate acid depositions even though no visible foliar injury developed. Foliar losses of nitrogen, calcium, magnesium and phosphorous increased with decreases in acid mist pH, whereas foliar potassium concentrations were unaffected by acid mist treatment.

ACIDIFICATION of precipitation as a result of fossil fuel combustion emissions has been reported in many areas of the world, particularly in Scandinavia and the Northeastern United States. The results of extensive pH and sulfate measurements show some instances of 200-fold increases in rainfall acidity since 1956 (Eriksson, 1963), with extreme acidity values of pH 2 recorded in fog (Walker, 1963) and pH 2.1 in rain (Likens and Bormann, 1974). The mechanisms by which sulfur dioxide is oxidized to sulfuric acid in the atmosphere are not well understood and there are many theories.

Foster (1969) indicated that atmospheric sulfur dioxide can rapidly oxidize to sulfuric acid in the presence of suspended metal oxide particulates (i.e., industrial dust) at high relative humidities. Increasing droplet acidities of pH 2–3 are associated with progressively smaller droplet diameters near coal-fired generating stations (Aynsley, 1970). Freiberg (1976) predicted that sulfur dioxide oxidation in the presence of iron oxide catalysts could form acid mist below pH 2 under certain environmental conditions. Cox and Penkett (1972) found photochemical oxidation of sulfur dioxide to acid mist possible in the presence of ozone and olefins in a polluted atmosphere. Photochemical activation of sulfur dioxide in the presence of nitrogen dioxide can also result in acid formation under high humidity conditions where dew is present (Bricard et al., 1971). Whatever formation mechanism predominates, acidified mist has a demonstrated phytotoxicity.

Visible symptoms of foliar injury characteristic of acid mist deposition have been described by Thomas, Hendricks, and Hill (1952) and Middleton, Darley, and Brewer (1957), while foliar histomorphological studies of acid mist injury have been performed by Gordon (1972) and Ferenbaugh (1976). Wood and Bormann (1974) demonstrated an acid mist effect on yellow birch growth and acid leachate effects on sugar maple and pinto bean nutrient cations (Wood and Bormann, 1975). However, no previous studies have examined acid mist effects on actual foliar macronutrient levels or specific full-term growth and reproductive yield of a flowering plant; therefore bush bean plant was selected in this experiment to provide general information about the effect of acid mist on plants.

MATERIALS AND METHODS—This experiment was designed to examine the effects of acid mist at various pH levels on the pathological and morphological development of bush bean (*Phaseolus vulgaris* L. cv. Contender).

Bush bean seeds were double sown in 140 plastic pots (10 cm) filled with Jiffy-Mix Plus, and later thinned to 1 sprout per pot. The potted plants were maintained in a greenhouse providing a 12-hour day at 20 ± 5 C, 40 ± 10% relative humidity and supplemental light of 11,000 lux. The plants were watered on alternate days and half-strength Hoagland nutrient solution was applied weekly. Seventy 2-week-old plants were randomly selected for treatment and tagged for identification. The plants were harvested at 8 weeks maturity after 6

[1] Received for publication 2 April 1979; revision accepted 6 August 1979.

The authors would like to express their appreciation to Ron Manabe and Stavros Howe for the tissue sulfur analysis and to Lou Grothaus for performing the statistical analysis. We appreciate the assistance and helpful suggestions of Lawrence Raniere, Ray Wilhour, Dan Krawczyk and Karen Randolph.

[2] Present address: U.S. Environmental Protection Agency, 200 S.W. 35th Street, Corvallis, OR 97330.

TABLE 1. *Foliar injury, vegetative and reproductive dry weight of bush bean plant exposed to acid aerosol*

	Acid mist pH					
	5.5	4	3	2.5	2	F values
Trifoliate injury[a]	0%	0%	0%	12%	23%	
Dry weight (gm) trifoliate leaves	4.03	3.46	3.28	2.79	2.71	
Percent reduction	0%	14%	19%	31%	33%	3.8**[b]
Dry weight (gm) including pods and seeds but without the root	12.67	11.61	10.63	8.92	8.73	
Percent reduction	0%	8%	16%	30%	31%	4.7**
Dry weight (gm) pod and seed formation	3.77	3.40	3.35	2.76	2.60	
Percent reduction	0%	10%	11%	28%	31%	3.1*
Total chlorophyll ($\mu g/cm^2$) trifoliate leaves	1.3	1.3	1.2	1.1	.9	
Percent reduction	0%	0%	8%	15%	31%	3.7*

[a] Mean of ten plant replications.
[b] Significance level: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

weekly, 45-min exposures to acid mist. A longer term exposure of another set of 40 plants was conducted for sulfur accumulation determination over the full 8-week plant life cycle on a 5-day-per-week schedule of 45-min treatments. Plant exposures were conducted in rotating-table exposure chambers where the mist was generated by sonic atomizing nozzles (Hindawi, Raniere, and Rea, 1976).

The mist solutions were prepared by dilution of analytical grade sulfuric acid with distilled water to five pH levels: 5.5, 4, 3, 2.5, and 2. Mineral additions were made in the same quantities as those determined from a 10-year average of bulk rainfall at Hubbard Brook, New Hampshire (Lee and Weber, 1976). Mist depositions at plant height were 0 (distilled water), .006, .06, .02, and .6 μg H_2SO_4/cm^2-min of mist. Dry collection deposition plates (Williams and Moser, 1976) and funneled rainfall gauges measured mist deposition at plant height. Potentiometric determination of H^+ ion concentration from the polyethylene rainfall gauges did not indicate any significant mist pH changes within the 45-min exposure period. The mean mist droplet diameter, determined by magnesium oxide coated slides (May, 1950), was 50 μm with a size range of 4 to 120 μm.

Symptoms of trifoliate leaf injury were evaluated at exposure termination. The chlorophyll concentrations were determined by acetone extraction (Gottschalk and Muller, 1966) on the trifoliates of 4 plants per treatment. The remaining 10 plants per treatment were freeze dried for dry weight and macronutrient determination. Total Kjeldahl nitrogen and total phosphorous samples were block digested with a liquid Kjeldahl digestant and concentrations determined colorimetrically with a Technicon Auto Analyzer (Jirka et al., 1976). Nitric and perchloric acid digested the potassium, calcium and magnesium tissue samples prior to determination by atomic absorption spectophotometry (W. L. Griffis, unpubl.). Sulfur concentration in plants from the long-term exposures were determined turbidimetrically after nitric and perchloric acid digestion (R. Manabe, pers. commun.).

RESULTS—Visual symptoms of foliar injury were observed on plant treatments where the acidity was less than pH 3. The primary visual injury progressed from incipient bronzed spotting, apparent within a few hours after mist deposition, to bifacial necrotic pitting by the following day. Gross leaf surface characteristics often influenced mist deposition to produce symptoms mimicking other pollutant effects. Pooling agglomerations of mist produced bleached interveinal injury resembling sulfur dioxide injury, while small flecks, not unlike moderate ozone injury, were observed where mist deposition was size-sorted on heavily pubescent leaves. Microscopic cross sections of the injured tissue showed the loss of chloroplast integrity and revealed the plasmolysis of the palisade cells. In severe cases, damage was apparent in the epidermis and spongy mesophyll as well.

A general decrease in plant vegetative and reproductive dry weight was observed with decreasing pH and increasing H_2SO_4 deposition (Table 1). Single factor analysis of variance indicated a significant acid effect ($P < 0.01$) across the pH gradient on the measured growth parameters. The trifoliate leaf dry weight appeared to be the most sensitive growth parameter recorded. Plants receiving the pH 2 treatment incurred a 33% trifoliate leaf dry weight reduction, while the pH 3 and 4 treatments produced greater than 10% reductions without any visible foliar injury. Pod dry weights illustrated much the same trend as the trifoliate weight with the greatest re-

TABLE 2. *Concentration of minerals in bush bean leaves exposed to acid mist*

Mineral[a] (ppm)	Acid mist pH				F Values
	5.5	4	3	2	
Nitrogen	39,200 ± 7,100	35,350 ± 8,240	32,400 ± 4,430	28,900 ± 7,370	3.7*[b]
Phosphorous	18,350 ± 3,340	16,540 ± 3,870	15,250 ± 3,020	13,582 ± 3,460	3.4*
Magnesium	15,200 ± 2,020	15,710 ± 2,060	11,131 ± 1,510	10,516 ± 1,200	22.1***
Calcium	29,530 ± 4,840	27,820 ± 4,830	21,300 ± 4,240	17,550 ± 3,130	14.4***
Potassium	26,270 ± 5,050	24,430 ± 4,210	25,830 ± 6,530	28,390 ± 6,600	.5
Sulfur	4,100 ± 1,020	4,434 ± 852	5,045 ± 1,007	8,133 ± 1,762	27.5***

[a] Mean of ten plant replications ± 1 SD.
[b] Significance level: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

ductions at the lower levels and approximately 10% dry-weight reductions at the intermediate pH levels. Analysis of variance did not indicate any nonrandom deviations in the mean number of pods produced at the various pH levels across the treatment gradient.

As acidity increased, a definite reduction ($P < 0.05$) in total foliar chlorophyll concentrations was observed across the pH gradient although the effect was not pronounced until pH 2.5 (Table 1). Differential susceptibility was not indicated between foliar chlorophyll *a* and *b*, even at the treatment extremes with a mean chlorophyll *a*:*b* ratio of 3.8. Table 2 illustrates the foliar reduction of most of the measured macronutrients as the result of increasing mist acidity. Significant losses in foliar nitrogen, phosphorous, magnesium and calcium were observed, while potassium concentrations appeared unaffected by acid treatment. Sulfur accumulations were decidedly higher in both the soil (Table 3) and foliar tissue of the low pH treatments as compared to the control. Soil calcium and potassium levels (Table 3) appeared unaffected by the pH treatments, which is in general agreement with Wood and Bormann's (1975) finding that soil cation levels apparently were not significantly affected by short term mist treatment.

DISCUSSION AND CONCLUSION—This experiment was performed to determine the response of bush bean plants to simulated acid mist at various pH levels. A foliar injury threshold of pH 2.5 was observed during this study. Similar visible symptoms of tissue damage have been produced with artificial mist of pH 3 or lower on kidney bean (Shriner, 1971), pinto bean, sugar maple and yellow birch (Wood and Bormann, 1974). The general similarities in broadleaf visible injury threshold suggest that relatively low pH levels are apparently required before symptomatic acid mist injury is visually apparent on broad foliage.

While the bush bean foliar injury was undoubtedly associated with the observed weight reductions at the intermediate pH treatment, significant reductions were apparent without detection of visible foliar injury symptoms. This suggests a mechanism other than simple photosynthetic reduction resulting from tissue destruction, especially considering the insignificant intermediate acid effect on foliar chlorophyll concentrations. Reductions in foliar macronutrient concentrations could possibly be associated with the observed weight reductions. Marked mineral leaching was recorded by Wood and Bormann (1975) with uninjured pinto bean foliage at pH 3.3. However, it is difficult to attribute the intermediate weight reductions to the foliar macronutrient concentration changes observed in this study since even the lowest recorded foliar macronutrient

TABLE 3. *Concentration of sulfur, calcium and potassium in soil of bush bean plants exposed to acid mist*

Mineral[a] (ppm)	Acid mist pH				F Values
	5.5	4	3	2	
Sulfur	1,850 ± 520	1,960 ± 410	2,126 ± 633	3,168 ± 628	6.7**[b]
Calcium	18,510 ± 3,449	18,600 ± 3,894	18,980 ± 4,367	17,960 ± 1,898	.11[NS]
Potassium	18,110 ± 2,214	17,620 ± 1,839	18,480 ± 2,416	18,240 ± 2,790	.17[NS]

[a] Mean of five replications ± 1 SD.
[b] Significance level: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
[NS] Not significant.

concentrations should have been adequate if the nutrient cations were present in an available form. Ferenbaugh's work (1974) suggested a H^+ ion-induced uncoupling of photophosphorylation as a significant mechanism of deleterious tissue acidification.

Reduced fruit set as a result of acid retardation of pollen tube elongation and pollen germination has been reported by Kratky et al. (1974). The 31% pod dry weight reduction at the low pH treatment of this study clearly illustrates an acid effect. However, another mechanism is possibly implicated; perhaps carbohydrate reductions are associated with trifoliate tissue reductions since there were no significant treatment differences in the number of pods produced. The ambiguities of the biophysiological mechanisms of acidified mist deserve further investigation.

Foliar reductions of nitrogen, calcium, phosphorous and magnesium increased with decreasing acid mist pH, whereas potassium concentrations were not affected. Mecklenburg, Tukey, and Morgan (1966) indicated foliar calcium loss was a passive process of ion exchange with the H^+ ion. Fairfax and Lepp (1975) and Tukey, Tukey, and Witter (1958) demonstrated that foliar potassium was less readily reduced by leaching than calcium. Rains, Schmid, and Epstein (1968) offered an alternative hypothesis, suggesting that the H^+ ion disrupts selective root mineral uptake resulting in decreased potassium levels available for leaching. Dahl and Skre (1971) found essentially the same effect, and postulated that acid precipitation could limit the availability of nutrients by reducing plant assimilation ability. The magnesium reductions observed may be related to acid conversion of the chlorophylls to phaeophytons and the resultant H^+ ion replacement of magnesium. The uniform reductions in both chlorophyll *a* and *b* across the pH gradient of this study agree with Ferenbaugh's (1974) findings in studies of *Phaseolus vulgaris*, where no differential chlorophyll susceptibility was indicated. Quantitative extrapolation of laboratory data to field conditions always requires considerable qualification. However, our general results should be applicable to qualitative considerations of acidified mist effects on plant macronutrient cycling, growth and productivity.

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